

AMENDMENTS TO THE CLAIMS

1. **(PREVIOUSLY PRESENTED)** A method of detecting DNA variation by monitoring the formation or dissociation of a complex consisting of:
 - (a) a single DNA strand of a double stranded DNA of at least 40 base pairs containing the locus of a variation, wherein said single DNA strand is within a monolayer of single DNA strands which are bound to a solid surface,
 - (b) an oligonucleotide or DNA analogue probe specific for one allele of the variation and capable of hybridizing to the single strand (a) to form a DNA duplex,
 - (c) an intercalating dye specific for the DNA duplex structure of (a) plus (b) which forms a complex with the said duplex and reacts uniquely when interacting within the DNA duplex,which method comprises:
 - (1) continually measuring an output signal indicative of interaction of the dye with duplex formed from the strand (a) and probe (b), and
 - (2) recording the temperature at which a change in reaction output signal occurs which is attributable to formation or dissociation of the complex and is thereby correlated with the strength with which the probe (b) has hybridized to the single strand (a).
2. **(ORIGINAL)** A method according to claim 1 including
 - (1) forming a series of two or more complexes of the kind defined, each with a probe specific for a different allele of the variation, and
 - (2) observing their respective denaturing or annealing conditions (e.g. denaturing or annealing temperatures) so as to distinguish alleles of the variation plus the homozygous or heterozygous state if appropriate.

3. **(ORIGINAL)** A method according to claim 1, in which the marker is one which fluoresces when intercalated in double stranded DNA.
4. **(ORIGINAL)** A method according to claim 3, in which the denaturing or annealing point is determined by reference to the first derivative of the fluorescence measurement curve.
5. **(ORIGINAL)** A method according to claim 3, in which denaturing or annealing point is determined by reference to the second derivative of the fluorescence measurement curve.
6. **(CANCELED)** A method according to claim 1, in which the single strand is attached to a support material.
7. **(PREVIOUSLY PRESENTED)** A method according to claim 1, in which the single strand is bound to the solid surface by a biotin/streptavidin type interaction.
8. **(ORIGINAL)** A method according to claim 1, in which the complex is formed by adding the probe and marker to the single strand in an appropriate buffer solution.
9. **(PREVIOUSLY PRESENTED)** A method according to claim 8, in which the buffer solution is Hepes buffer having a salt concentration less than 200 mM.
10. **(ORIGINAL)** A method according to claim 1, using a fluorescent intercalating dye, in which the dye is SYBR Green I.
11. **(PREVIOUSLY PRESENTED)** A method according to claim 1, in which double stranded DNA is a product of PCR amplification of a target sequence.

12. **(ORIGINAL)** A method according to claim 11, in which the PCR product is at least 100 base pairs in length.

13. **(ORIGINAL)** A method according to claim 11, in which the PCR product is from 40 to 100 base pairs in length.

14. **(PREVIOUSLY PRESENTED)** A method of detecting DNA variation which comprises bringing together:

- (a) a single DNA strand of a double stranded DNA of at least 40 base pairs containing the locus of a variation, wherein said single DNA strand is within a monolayer of single DNA strands which are bound to a solid surface,
- (b) an oligonucleotide or DNA analogue probe specific for one allele of the variation and capable of hybridizing to the single strand (a) to form a DNA duplex,
- (c) an intercalating dye specific for the DNA duplex structure of (a) plus (b) which forms a complex with the said duplex and reacts uniquely when interacting within the duplex,

thereby forming a complex consisting of the components (a), (b) and (c), wherein the components (a), (b), and (c) are brought together under conditions in which either

- (i) the component (a) hybridizes to component (b) and the complex is formed with component (c), or
- (ii) the components (a) and (b) do not hybridize and the complex with component (c) is not formed,

- (2) thereafter steadily and progressively adjusting the temperature, respectively, either
 - (i) to denature the formed DNA duplex and cause dissociation of the complex, or
 - (ii) to cause formation of the DNA duplex and resulting complex,
- (3) continually measuring an output signal indicative of the extent of hybridization of (a) and (b) and resulting complex formation with (c), and
- (4) recording the temperature at which a change of output signal occurs which is indicative of, respectively,
 - (i) dissociation of the complex, or
 - (ii) formation of the complex.

15. **(ORIGINAL)** A method according to claim 14 which comprises
 - (1) forming a series of two or more complexes of the kind defined, each with a probe specific for a different allele of the variation, and
 - (2) observing their respective denaturing or annealing conditions (e.g. denaturing or annealing temperatures) so as to distinguish alleles of the variation plus the homozygous or heterozygous state if appropriate.
16. **(ORIGINAL)** A method according to claim 14, in which the marker is one which fluoresces when intercalated in double stranded DNA.
17. **(ORIGINAL)** A method according to claim 16, in which the denaturing or annealing point is determined by reference to the first derivative of the fluorescence measurement curve.
18. **(ORIGINAL)** A method according to claim 16, in which denaturing or annealing point is determined by reference to the second derivative of the fluorescence measurement curve.
19. **(CANCELED)** A method according to claim 14, in which the single strand is attached to a support material.
20. **(PREVIOUSLY PRESENTED)** A method according to claim 14, in which the single strand is bound to the solid surface by a biotin/streptavidin type interaction.
21. **(ORIGINAL)** A method according to claim 14, in which the complex is formed by adding the probe and marker to the single strand in an appropriate buffer solution.
22. **(PREVIOUSLY PRESENTED)** A method according to claim 21, in which the buffer solution is Hepes buffer having a salt concentration less than 200 mM.

23. **(ORIGINAL)** A method according to claim 14, using a fluorescent intercalating dye, in which the dye is SYBR Green I.
24. **(PREVIOUSLY PRESENTED)** A method according to claim 14, in which the double stranded DNA is a product of PCR amplification of a target sequence.
25. **(ORIGINAL)** A method according to claim 24, in which the PCR product is at least 100 base pairs in length.
26. **(ORIGINAL)** A method according to claim 24, in which the PCR product is from 40 to 100 base pairs in length.
27. **(PREVIOUSLY PRESENTED)** A method of detecting DNA variation which comprises:
 - (1) forming a complex consisting of:
 - (a) a single DNA strand of a double stranded DNA of at least 40 base pairs containing the locus of a variation, wherein said single DNA strand is within a monolayer of single DNA strands which are bound to a solid surface,
 - (b) an oligonucleotide or DNA analogue probe specific for one allele of the variation hybridized to the single strand (a) to form a duplex, and
 - (c) an intercalating dye specific for the DNA duplex structure of (a) plus (b) and which reacts uniquely when interacting within the DNA duplex, and
 - (2) continually measuring an output signal of the extent of the resulting reaction of the marker and the duplex while steadily increasing the temperature,
 - (3) recording the temperature at which a change in reaction output signal occurs which is attributable to dissociation of the complex and is thereby correlated with the strength with which the probe (b) has hybridized to the single strand (a).

28. **(ORIGINAL)** A method according to claim 27, which comprises

- (1) forming a series of two or more complexes of the kind defined, each with a probe specific for a different allele of the variation, and
- (2) observing their respective denaturing or annealing conditions (e.g. denaturing or annealing temperatures) so as to distinguish alleles of the variation plus the homozygous or heterozygous state if appropriate.

29. **(ORIGINAL)** A method according to claim 27, in which the marker is one which fluoresces when intercalated in double stranded DNA.

30. **(ORIGINAL)** A method according to claim 29, in which the denaturing or annealing point is determined by reference to the first derivative of the fluorescence measurement curve.

31. **(ORIGINAL)** A method according to claim 29, in which denaturing or annealing point is determined by reference to the second derivative of the fluorescence measurement curve.

32. **(CANCELED)** A method according to claim 27, in which the single strand is attached to a support material.

33. **(PREVIOUSLY PRESENTED)** A method according to claim 27, in which the single strand is bound to the solid surface by a biotin/streptavidin type interaction.

34. **(ORIGINAL)** A method according to claim 27, in which the complex is formed by adding the probe and marker to the single strand in an appropriate buffer solution.

35. **(PREVIOUSLY PRESENTED)** A method according to claim 34, in which the buffer solution is Hepes buffer having a salt concentration less than 200 mM.

36. **(ORIGINAL)** A method according to claim 27, using a fluorescent intercalating dye, in which the dye is SYBR Green I.
37. **(PREVIOUSLY PRESENTED)** A method according to claim 27, in which the double stranded DNA is a product of PCR amplification of a target sequence.
38. **(ORIGINAL)** A method according to claim 37, in which the PCR product is at least 100 base pairs in length.
39. **(ORIGINAL)** A method according to claim 37, in which the PCR product is from 40 to 100 base pairs in length.

40. **(PREVIOUSLY PRESENTED)** A method of detecting DNA variation which comprises:

- (1) bringing together:
 - (a) a single DNA strand of a double stranded DNA of at least 40 base pairs containing the locus of a variation, wherein said single DNA strand is within a monolayer of single DNA strands which are bound to a solid surface,
 - (b) an oligonucleotide or DNA analogue probe specific for one allele of the variation and capable of hybridizing to the single strand (a) to form a DNA duplex,
 - (c) an intercalating dye specific for the DNA duplex structure of (a) plus (b) and which reacts uniquely when interacting within the duplex, the components (a), (b) and (c) being brought together prior to formation of the defined complex and under conditions in which (a) and (b) do not hybridize;
- (2) steadily adjusting the temperature to cause formation of the duplex and resulting complex consisting of components (a), (b), and (c), and
- (3) measuring an output signal indicative of the occurrence of hybridization of (a) and (b) (herein termed the annealing point).

41. **(ORIGINAL)** A method according to claim 40, which comprises

- (1) forming a series of two or more complexes of the kind defined, each with a probe specific for a different allele of the variation, and
- (2) observing their respective denaturing or annealing conditions (e.g. denaturing or annealing temperatures) so as to distinguish alleles of the variation plus the homozygous or heterozygous state if appropriate.

42. **(ORIGINAL)** A method according to claim 40, in which the marker is one which fluoresces when intercalated in double stranded DNA.

43. **(ORIGINAL)** A method according to claim 42, in which the denaturing or annealing point is determined by reference to the first derivative of the fluorescence measurement curve.
44. **(ORIGINAL)** A method according to claim 42, in which denaturing or annealing point is determined by reference to the second derivative of the fluorescence measurement curve.
45. **(CANCELED)** A method according to claim 40, in which the single strand is attached to a support material.
46. **(PREVIOUSLY PRESENTED)** A method according to claim 40, in which the single strand is bound to the solid surface by a biotin/streptavidin type interaction.
47. **(ORIGINAL)** A method according to claim 40, in which the complex is formed by adding the probe and marker to the single strand in an appropriate buffer solution.
48. **(PREVIOUSLY PRESENTED)** A method according to claim 47, in which the buffer solution is Hepes buffer having a salt concentration less than 200 mM.
49. **(ORIGINAL)** A method according to claim 40, using a fluorescent intercalating dye, in which the dye is SYBR Green I.
50. **(PREVIOUSLY PRESENTED)** A method according to claim 40, in which the double stranded DNA is a product of PCR amplification of a target sequence.
51. **(ORIGINAL)** A method according to claim 50, in which the PCR product is at least 100 base pairs in length.

52. **(ORIGINAL)** A method according to claim 50, in which the PCR product is from 40 to 100 base pairs in length.

53-66. **(CANCELED)**

67. **(PREVIOUSLY PRESENTED)** A method according to claim 4 comprising determining the presence, in the negative of said derivative of the fluorescent measurement curve, one or both of: a first peak associated with the denaturing of a match probe target duplex and a second peak associated with the presence of a mis-match probe target duplex.

68. **(PREVIOUSLY PRESENTED)** A method according to claim 17 comprising determining the presence, in the negative of said derivative of the fluorescent measurement curve, one or both of: a first peak associated with the denaturing of a match probe target duplex and a second peak associated with the presence of a mis-match probe target duplex.

69. **(PREVIOUSLY PRESENTED)** A method according to claim 30 comprising determining the presence, in the negative of said derivative of the fluorescent measurement curve, one or both of: a first peak associated with the denaturing of a match probe target duplex and a second peak associated with the presence of a mis-match probe target duplex.

70. **(PREVIOUSLY PRESENTED)** A method according to claim 43 comprising determining the presence, in the negative of said derivative of the fluorescent measurement curve, one or both of: a first peak associated with the denaturing of a match probe target duplex and a second peak associated with the presence of a mis-match probe target duplex.

71. **(PREVIOUSLY PRESENTED)** A method of detecting DNA variation by monitoring the formation or dissociation of a complex consisting of:

- (a) a single DNA strand of a double stranded DNA of at least 40 base pairs containing the locus of a variation, bound within a two dimensional monolayer on the surface of a solid support,
- (b) an oligonucleotide or DNA analogue probe specific for one allele of the variation and capable of hybridizing to the single strand (a) to form a DNA duplex, and;
- (c) an intercalating dye specific for the DNA duplex structure of (a) plus (b) which forms a complex with the said duplex and reacts uniquely when interacting within the DNA duplex,

which method comprises:

- (1) continually measuring an output signal indicative of interaction of the dye with duplex formed from the strand (a) and probe (b), and
- (2) recording the temperature at which a change in reaction output signal occurs which is attributable to formation or dissociation of the complex and is thereby correlated with the strength with which the probe (b) has hybridized to the single strand (a).

72. **(PREVIOUSLY PRESENTED)** The method of claim 71 wherein the single DNA strand is bound to the surface of the support by a biotin/streptavidin type interaction.

73. **(PREVIOUSLY PRESENTED)** The method of claim 71 wherein the complex is formed by adding the probe and the marker to the single strand in a buffer having a salt concentration less than 200mM.

74. **(PREVIOUSLY PRESENTED)** A method of detecting DNA variation by monitoring the formation or dissociation of a plurality of complexes, each said complex consisting of:

- (a) a single DNA strand of a double stranded DNA of at least 40 base pairs containing the locus of a variation,
- (b) an oligonucleotide or DNA analogue probe specific for one allele of the variation and capable of hybridizing to the single strand (a) to form a DNA duplex, and;
- (c) an intercalating dye specific for the DNA duplex structure of (a) plus (b) which forms a complex with the said duplex and reacts uniquely when interacting within the DNA duplex,

wherein each said complex is bound to a surface of a solid support and said plurality of complexes form a monolayer on said surface,

which method comprises:

- (1) continually measuring an output signal indicative of interaction of the dye with duplex formed from the strand (a) and probe (b), and
- (2) recording the temperature at which a change in reaction output signal occurs which is attributable to formation or dissociation of the complex and is thereby correlated with the strength with which the probe (b) has hybridized to the single strand (a).

75. **(PREVIOUSLY PRESENTED)** The method of claim 74 wherein the single DNA strand is bound to the surface of the support by a biotin/streptavidin type interaction.

76. **(PREVIOUSLY PRESENTED)** The method of claim 74 wherein the complex is formed by adding the probe and the marker to the single strand in a buffer having a salt concentration less than 200mM.